

Standard Operation Procedure (S.O.P.) for Plasmid Purification using MAXIprep Kit

S.O.P. prepared by Andrea Rodríguez, 2021

Modified by José E Lizardi Ortiz, Ph.D., 2021

This S.O.P. will direct you in the procedure of plasmid purification through the QIAGEN QIAprep Spin MAXIprep Kit (cat.no. 12163). This method provides a high yield of ultrapure super coiled plasmid DNA useful for transfection, in vitro transcription and translation, enzymatic modifications and digestion, sequencing, ligation, and transformation, among others. This kit allows purification of up to 500 µg of high-copy and low-copy plasmids up from overnight culture. Special modifications not considered in this SOP must be taken in account when purifying low-copy vectors and cosmids, large plasmids (>45–50 kb), and DNA prepared using other methods.

Different factors could affect plasmid purification, including

- Plasmid size: larger plasmids reduce elution efficiency.
- Copy number: plasmid varies in copy number depending on the origin of replication and size. The protocol could be modified to purify low
- Host strain: certain strains inhibit enzyme activities and yield lower quality DNA. The strains DH1, DH5α, C600, Top10, and XL1-Blue yield high-quality DNA. HB101, TG1 and JM100 contain large amount of carbohydrites that can inhibit enzyme activities and high levels of endonucleases yielding low DNA quality.
- Culture composition and volume: larger culture volumes reduce the biomass-to-lysis buffer ration reducing recovery yield and purity. This SOP is developed to a growth media (Miller LB broth), specific volume, and incubation time (12–16 hours) to reach ~3–4 10⁹ cell/mL.
- Environment salt levels
- pH levels

Important:

- **This protocol is designed for high-copy plasmid with size <45 kb. Modifications must be introduced to purify low-copy vectors or large plasmids >45–50 kb and they must be determined for each plasmid. REFER TO THE MANUAL FOR RECOMMENDATIONS. This protocol could not be used for very-low copy plasmids (<10 copies/cell).**
- **If you are working with HB101 strain or its derivatives, consult the manual for recommendations.**
- **Bacterial cell culture should be no larger than 100 mL.**
- **Do not shake the buffers vigorously.**
- **Check RNase A and LyseBlue was added to Buffer P1 (a box in the cap should be checked). Buffer P1 has a life span of 1 year after RNase A addition and must be stored at 4 °C.**
- **Check Buffer P2 for salt precipitation. If that is the case, warm the buffer at 37 °C.**
- **Pre-chill Buffer P3 at 4°C before using.**
- **Close the buffer bottles immediately after use to avoid acidification by CO₂.**
- **Resuspension of DNA after isopropanol/ethanol precipitation can be carried by water or Tris-Cl buffer, pH 8.5. Avoid using Tris–EDTA (TE) buffers if the purpose of the purification is not long-term storage. If the DNA will be further employed in enzymatic reactions is preferable to elute in water. If water is use, store at –20 °C (preferably at –80 °C) if no immediate use is expected.**
- **All tips and microtubes must be autoclaved.**
- **All volumes are given on a per culture tube basis.**

Materials:

- 70% Ethanol
- Waste Container with Bleach (2% Sodium Hypochlorite)
- Inoculation Loops
- 5 mL Micropipette and Tips (in case there is no 5 mL micropipettes, you can use 5 mL or 10 mL serological pipets)
- 5-, 10-, 25-, and 50-mL Serological Pipet
- 1000 µL Micropipette and Tips
- 14 mL Round-Bottom Culture Tubes
- 250 mL Erlenmeyer Flask
- 250 mL Centrifuge Bottle
- 50 mL Oak Ridge Centrifuge Tubes with Cap (Clear Tube, Polycarbonate)
- 1.5 mL Microtubes
- Miller LB Media with the Appropriate Antibiotics and Chemicals (1% Tryptone, 0.5% Yeast Extract, 1% NaCl)
- Buffer P1 (stored at 4 °C)
- Buffer P2 (stored at room temperature)
- Buffer P3 (stored at room temperature)
- Buffer QBT (stored at room temperature)
- Buffer QC (stored at room temperature)
- Buffer QF (stored at room temperature)
- 100% Isopropanol
- Nuclease-free Water or TE Buffer (10 mM Tris-Cl, pH 8.0; 1 mM EDTA; stored at room temperature)
- QIAGEN-tip 500
- High-Speed Refrigerated Centrifuge for 50 mL Oak Ridge Tubes
- Refrigerated Centrifuge for 250 mL Centrifuge Bottle
- Incubator with Orbital Shaker at 37 °C and 225 r.p.m.
- Vortex

Procedure:

Day Before:

- a) The working area should be sanitized using 70% ethanol prior to placing any materials on the bench.
- b) Prepare the waste container with concentrated bleach for decontamination. ***Any material that is in contact with bacteria must be decontaminated in this container for at least 30 minutes.***
- c) Determine how many culture tubes will be purified and label the tubes properly. In each culture tube (14 mL tube), add 2 mL of LB media containing the appropriate antibiotic and chemicals and inoculate the media with the target bacteria from an agar plate or glycerol stock using an inoculation loop. ***If the source of the bacteria is cold, use cold media during inoculation. If the source is at 37 °C, pre-warm the media to the same temperature***
- d) Incubate the culture for ~8 hours in an orbital shaker incubator at 37 °C, 225 r.p.m. The samples should look turbid, indicating bacterial growth.
- e) Prepare a 1/500 dilution of the initial bacterial culture in fresh LB media containing the appropriate antibiotics and chemicals by mixing 100 mL of fresh LB media with antibiotics and

chemicals (*pre-warmed at 37 °C*) and 200 µL of initial culture in a 250 mL Erlenmeyer flask. Incubate for ~16 hours at 37 °C, 225 r.p.m. in an orbital shaker incubator. The samples should look turbid.

- f) Carefully invert the remaining bacterial culture into the waste container with bleach and let the bleach solution to rest for at least 30 minutes. Dispose the solution into the proper waste container and discard the materials in a biological waste (trash can with red bag).
- g) The working area and any material should be sanitized using 70% ethanol at the end.

Purification Process:

- a) The working area should be sanitized using 70% ethanol prior to placing any materials on the bench.
- b) Prepare the waste container with concentrated bleach for decontamination. **Any material that is in contact with intact bacteria must be decontaminated in this flask for at least 30 minutes.**
- c) Properly label the 50 mL Oak Ridge centrifuge tubes, the 1.5 mL microtubes to be used for DNA elution, and the QIAGEN-tip 500 column. Set the column in the proper stand and cover the top opening with aluminum foil or paraffin. Place the **Buffer P2** at 37 °C to dissolve any solid, prechill the **Buffer P3** in ice or 4 °C, and prewarm 300 µL of water to be used during elution at 37 °C.
- d) Transfer the 100 mL of culture media into a 250 mL centrifuge bottle and centrifuge at 6,000 x g (preferably at 4°C) for 15 minutes to harvest the bacterial cells. Use this time to place a medium-size piece of aluminum foil on the bench and cover it with a layer of paper towel before centrifuge is done.
 - o In case you are purifying one sample or an odd number of samples, fill a second centrifugation bottle with 100 ml of water to balance rotor.
- e) Remove the supernatant by carefully inverting the centrifuge bottle into the waste container with bleach and wait until almost all culture media has been transferred into the waste container. Place the culture tube upside down on the paper towel to get rid of final drops and allow it to dry.
- f) Resuspend bacterial pellet by adding 10 mL of **Buffer P1** into the 250 mL centrifuge bottle using a 10 mL serological pipet and apply vortex until bacterial pellet is completely resuspended. Briefly centrifuge the bottle at 500 x g to remove drops from the bottle's walls and transfer the resuspension into a 50 mL Oak Ridge centrifugation tube.
- g) Add 10 mL of **Buffer P2** to the tube and mix by inverting the sealed tube 4-6 times. The solution should turn blue if LyseBlue is present. Mixing should result in homogeneously colored suspension. Incubate at room temperature for 5 minutes.
 - o **DO NOT VORTEX.** This can result in shearing of genomic DNA. **Do not allow the lysis to proceed for more than 5 minutes.**
- h) Add 10 mL of pre-chilled **Buffer P3** to the tube and mix by inverting it 4-6 times. The suspension should produce a cloudy white precipitation, keep carefully inverting until solution has no trace of blue color. Incubate on ice (or 4°C) for 20 minutes.
 - o Using pre-chilled buffer P3 optimizes results.
 - o The precipitate material contains genomic DNA, protein, cell debris, and potassium dodecyl sulfate.
- i) Centrifuge the tube at 30,000–35,000 x g for 30 min at 4°C. A white pellet will be formed at the

walls and bottom of the tube. Carefully transfer the DNA-containing supernatant to a new 50 mL Oak Ridge centrifuge tube using a 10 mL serological pipet and centrifuge again at 30,000–35,000 $\times g$ for 30 min at 4°C.

- In case you are purifying one sample or an odd number of samples, fill a second centrifugation bottle with similar volume of water to balance rotor.
 - **Avoid touching the white pellet while removing the supernatant.**
- j) During the centrifuge procedure, equilibrate the QIAGEN-tip 500 by applying 10 mL of **Buffer QBT** and allow the column to completely empty by gravity flow. The original waste container can be used to recollect this buffer. Wait until all liquid has been emptied from the column.
- k) Transfer the DNA-containing supernatant into the QIAGEN-tip 500 using a 10 mL serological pipet and allow it to enter the resin by gravity flow. The DNA should be contained in the column and the buffer will pass through.
- l) Wash the QIAGEN-tip 500 with 30 ml of **Buffer QC**. Allow the buffer to move through the column by gravity flow. Let the buffer go into the waste container.
- m) Wash again the QIAGEN-tip 500 with 30 ml of **Buffer QC**. Allow the buffer to move through the column by gravity flow. Let the buffer go into the waste container.
- n) Place a 50 mL Oak Ridge centrifuge tube at the bottom of the QIAGEN-tip 500 and elute DNA with 15 ml of **Buffer QF**.
- o) Precipitate the DNA by adding 10.5 mL of room-temperature isopropanol to the elute DNA. Mix and centrifuge immediately at 30,000–35,000 $\times g$ for 30 min at 4°C to prevent overheating.
- p) Identify the DNA pellet and mark the outside of the centrifuge tube to visualize the area containing the pellet in subsequent steps. Carefully remove the supernatant using a 10 mL serological pipet. **Keep in mind that the pellet may be very fine and not visible, but the DNA is on the inner walls of the tube.**
- q) Wash the DNA pellet with 5 ml of 70% ethanol and centrifuge at 30,000–35,000 $\times g$ for 30 min at 4°C. Carefully remove the supernatant without disturbing the pellet using a 5 mL serological pipet. Let the pellet dry for 10 minutes at 37 °C before next step.
- r) Redissolve the DNA in 250 μ l of prewarm ultrapure water (37 °C) and transfer into appropriately labeled 1.5 ml microtube. Store sample at 4 °C if it will be used in short time (<2 weeks).
- If long-term storage is required, aliquot and place the stock at –80 °C.
 - The decision between **water** and **Tris-Cl Buffer** will depend on the subsequent use of the plasmid DNA. If you expect to perform subsequent experiments with the plasmid, it is recommendable to use **water** as the **Tris-Cl Buffer** contains salts which could affect other experiments such as restriction enzyme digestion.
 - If the sole purpose of the purification is long-term storage, it is recommended to use TE buffer, which provide better stabilization of DNA. **Label the microtube to notify that TE is used.**
 - **Avoid freeze and thaw cycles because degrades DNA.**
- s) Carefully invert the remaining bacterial culture into the waste container with bleach and let the bleach solution to rest for at least 30 minutes. Dispose the solution using the proper waste container and discard the materials in a biological waste (trash can with red bag).

- t) The working area and any material should be sanitized using 70% ethanol at the end. Return any material and equipment to its proper place.
- u) Be sure all equipment is shut down: centrifuges and incubator. The pipettes must be returned to their maximal volume.